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Glucuronic Acid Production *in vitro* by Liver Slices from Rats Fed Dimethylaminoazobenzene (Butter Yellow).

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Surviving liver slices, when shaken in a saline phosphate medium (pH 7.4) in the Warburg apparatus, produce conjugated glucuronic acids in the presence of borneol, menthol or phenol.[1, 2] This production has been shown to be significantly increased in the presence of sodium lactate or sodium pyruvate.[1] Beuding and Ladewig[2] reported, furthermore, that the power of liver to conjugate borneol is reduced in severe phosphorus poisoning and is not affected in fatal chloroform poisoning.

The experiments here reported were undertaken to determine whether or not the capacity of rat livers to conjugate borneol with glucuronic acid in the presence of sodium lactate is impaired by the oral administration of dimethylaminoazobenzene, a compound which causes severe cirrhosis and malignant neoplasms of the liver of the rat.[3] The addition of 15% yeast to the basal diet has been shown to give complete protection against both types of liver change.[4, 5] Stevenson, Dobriner and Rhoads[6] have provided evidence that orally administered dimethylaminoazobenzene is split at the azo link in the course of its breakdown in the animal body, and that conjugated para-aminophenol is excreted in the urine. Para-aminophenol has been shown to be conjugated with glucuronic acid and sulfate *in vivo*.[7]

The method used to measure glucuronide formation *in vitro* was essentially that described by Lipschitz and Beuding.*[1] The assays of glucuronide in the urine were made by the method of Maughan, Evelyn and Browne.[8] The standard used was a sample of pure sodium pregnandiol glucuronide supplied through the courtesy of

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1 Lipschitz, W., and Beuding, E., *J. Biol. Chem.*, 1939, **129**, 333.

2 Beuding, E., and Ladewig, P., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 433.

3 Kinosita, R., *Trans. Jap. Path. Soc.*, 1937, **27**, 665.

4 Ando, T., *Gann.*, 1938, **32**, 252.

5 Sugiura, K., and Rhoads, C. P., *Cancer Research*, 1941, **1**, 1.

6 Stevenson, E., Dobriner, K., and Rhoads, C. P., in press.

7 Jaffee, M., *Z. f. Physiol., Chemie.*, 1888, **12**, 295.

* A Pfaltz & Bauer photoelectric colorimeter was used.

8 Maughan, G. B., Evelyn, K. A., and Browne, J. S. L., *J. Biol. Chem.*, 1938, **126**, 567.

Dr. Browne. The urine used was collected over 3- to 4-day periods, from groups of 5 rats each. The diets used were the same as those previously described.[9]

The results of the experiments (Table I) indicate that the feeding of dimethylaminoazobenzene causes no impairment of the ability of liver slices to conjugate d,l borneol *in vitro*. Even livers which were markedly cirrhotic and contained hepatomas or cholangiomas gave normal or somewhat elevated values.

The results of the study of the urinary excretion of glucuronides by rats fed dimethylaminoazobenzene are summarized in Table II. Animals fed this compound excreted more glucuronides in the urine than did the rats fed the control diets. The amount of glucuronic acid found in the urine of rats which had ingested the chemical for over 100 days was found to be low, possibly due to the decreased intake of food containing the compound. The in-

TABLE I.
d,l Borneol Conjugation with Glucuronic Acid by Liver Slices *in Vitro* in the Presence of Sodium Lactate.

Group	No. of days on diet	No. of rat livers assayed	Mg glucuronic acid formed per g liver 90 min. Variation	Avg value
Normal diet		8	.9-2.8	1.5
Basal diet	30-100	13	.8-3.4	2.0
Basal diet + dimethylaminoazobenzene	30-150	15	1.3-5.3	3.0
+ Dimethylaminoazobenzene + 15% Yeast	80-130	10	1.1-4.0	2.1
+ Dimethylaminoazobenzene + Non-protective Supplement	50-150	20	1.5-6.7	3.1

TABLE II.
Urinary Output of Glucuronides by Rats.

Diet	Days on diet (Interval studied)	No. Collections	Mg glucuronic acid per rat day Variations	Avg
Normal		10	1.1-10.0	5.2
Basal	1-52	10	3.8-15.0	6.1
Basal + 6 mg Dimethylaminoazobenzene (1)	1-40	12	8.8-22.2	12.9
+ 6 mg Dimethylaminoazobenzene (2)	1-32	10	9.2-18.9	13.2
+ 6 mg Dimethylaminoazobenzene (3)	100-160	20	.8-5.8	3.2
+ Dimethylaminoazobenzene + 15% Yeast	1-40	13	7.1-20.0	12.9
+ Dimethylaminoazobenzene + 15% Yeast	100-140	11	6.5-19.8	10.5

[9] Kensler, C. J., Sugiura, K., and Rhoads, C. P., *Science*, 1940, **91**, 623.

gestion of food by rats in the early stages of dimethylaminoazobenzene feeding and of rats receiving a protective supplement is approximately 10 g per day per rat. Rats supplied the unsupplemented carcinogenic diet eat between 4 and 6 g per day when they have been on this diet for 100 days or more. Another contributing factor probably is that late in the experiment less aminophenol or other phenolic compounds are available for conjugation with glucuronic acid due to some metabolic change associated with the damage to the liver cells.

Conclusions. 1. The ability of rat liver slices to conjugate borneol with glucuronic acid *in vitro* is not impaired, even when marked cirrhosis and malignant degeneration of the liver are produced, by the feeding of dimethylaminoazobenzene. 2. The glucuronide excretion of rats fed dimethylaminoazobenzene is initially somewhat higher than in the control animals. However, after 100 days of dimethylaminoazobenzene feeding, the rate is less than 25% of that seen initially.

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Experimental Hypertension from Section of Moderator Nerves: Relationship to Presence of Kidney Tissue.*

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Hering,[1] Koch and Mies,[2] Heymans, Bouckaert and Regniers[3] and others have shown that section of both carotid sinus nerves and both aortic depressor nerves in experimental animals results in an immediate rise in blood pressure. Section of these 4 moderator nerves in dogs results in persistent hypertension which is essentially neurogenic in origin. Following total sympathectomy, this form of hypertension disappears, according to Heymans and Bouckaert.[4]

* This work was aided by a grant from the Commonwealth Fund for the study of essential hypertension.

1 Hering, H. E., *Die Karotissinusreflexe auf Herz und Gefasse,* Dresden, Th. Steinkopff, 1927.

2 Koch, E., and Mies, H., *Krankheitsforschung,* 1929, **7**, 241.

3 Heymans, C., Bouckaert, J. J., and Regniers, P., *Le Sinus carotidien,* Paris, 1933.

4 Heymans, C., and Bouckaert, J. J., *Bull. de l'Acad. Royale de Med. de Belg.,* 1936, **6**, 42.